The Growth and Cultural Characteristics of Pathogenic and Non-pathogenic Monilia on the Chorio-allantoic Membrane of the Chick Embryo

by

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        1. <u>Monilia albicans</u>

a. Strain 1	Obtained from culture from lesion on hand.
b. Strain 2	Obtained from culture of peritoneal cavity.

The present thesis is based on a study of the growth characteristics of pathogenic and non-pathogenic Monilia on the chorio-allantoic membrane of the chick. The gross and histological appearance of the growth obtained and the lesions produced by the Monilia are described. The types of reaction produced on the chorio-allantoic membrane by three different species of Monilia are compared.

REVIEW OF LITERATURE

As far as is known the embryologists were the first to use fertile incubating hen's eggs. They recognized this medium as a convenient, easily adaptable and constantly available source in which to study developmental anatomy. Goodpasture(13)states that Beguelin(2)was the first to make a window in the egg shell so that the growing embryo could be observed during its development. Beguelin's method was to remove the shell and its membrane from the blunt end of the hen's egg during the early days of incubation. When the egg was not being studied, he would place over the opening a piece of shell which had been cut from the blunt end of another egg. Using this technic it was possible for him to remove the cover and so make observations whenever necessary. This work was re-

ported before the Berlin Academy in 1749.

During the nineteenth century several other workers used similar methods and Scymkiewicz,(24) in 1815, had the ingenuity to cover the window in the shell by a coverglass, which was sealed with "wax" so that he was able to watch the developing embryo without disturbing it.

Gerlach,(8) in 1886, invented an instrument that he called the embryoscope. It could be fixed in the shell opening and contained a removable piece of glass which allowed him to operate upon the growing embryo or to observe it through a microscope. The instrument was clumsy and never came into general use.

In 1898, Florence Peebles(21) also made windows in the shell thus permitting her to injure various portions of the primitive streak and to observe the subsequent development of the embryo. The method used by Peebles was a modification of Gerlach's technic.

It is interesting to note that the investigators mentioned above were all interested in problems of embryology and that any infection, which might by chance result from their manipulation of the embryo, would terminate or hinder their experiments.

Although egg contents have been employed for many years in the composition of media for bacteria, they were seldom used in making media for fungi. However, isolated early attempts were made to use the egg as a medium for the cultivation of fungi, for Wolff and Israel,(26) in 1891, injected purulent material removed from a retromaxillary nodule into raw and partially boiled hen's and pigeon's eggs and secured pure cultures of <u>Actinomyces</u>.

Levaditi,(15) in 1906, is generally credited with the first use of the developing chick embryo for the study of infection. In 1905, Borrel, according to Levaditi, injected into fertile eggs a small quantity of chicken blood containing spirilla of fowls and found that an acute septicemia, caused by the spirilla, of the embryo took place. These results were apparently not published by Borrel but in continuing the experiments, Levaditi perforated the shell of the egg with a needle, injected infected blood into the albumen of the egg and sealed the puncture hole. The striking thing about this experiment was that the spirilla would not grow unless the egg was fertile and contained a developing embryo. In other words, the presence of living embryonic tissue appeared to be necessary

in order that infection by the spirillum could take place.

Rettger,(22) in 1913, studied the bacteriology of the hen's egg with special reference to its freedom from bacterial infection and found that the egg contents remained a sterile medium unless the eggs were subjected to moisture and dirt.

Murphy(20) was able successfully to graft the cells of certain mammalian tumors on to the chorio-allantoic membrane and was even able to transplant tumors from membrane to membrane. However, he was unable to get a successful transplant when newly hatched chicks were used. This experiment indicated that so far as tumors are concerned a change from a susceptible to an insusceptible state of the embryo occurred during its last two days of life within the shell. Stevenson,(25) however, attempted to graft human tumors on to the chick membrane without success.

Rous and Murphy(23) in studying the virus of the Rous' sarcoma of chickens demonstrated the value of the chorio-allantoic membrane of fertile eggs as a medium for experimental pathological problems as these investigators were able to produce growth of the sarcoma on the membrane.

Juan and Straub(14) in 1920 were able successfully to infect chick

embryos with the virus of avian pest. The inoculation was into the yolk sac and they were able to obtain six positive passages, but subsequent passages were negative.

Clark,(5) in 1920, in looking for a method by which his students in embryology could study problems which involved operations on chick embryos described the present sterile technic for making windows in the egg shell through which the embryo and its membranes could be observed and manipulated. This method has served as the foundation for the technic used by all later investigators.

In 1923, mention was made by Askanazy(1) of the production of tuberculous chicks by the infection of fertile eggs but little was done toward the development of the method. In 1929, Gay and Thompson(7) inoculated vaccinia virus into the yolk sac and recovered the virus in the second generation but the third transfer failed. In 1931, Woodruff and Goodpasture(27) published a paper on the susceptibility of the chorio-allantoic membrane of chick embryos to infection with fowl-pox virus modifying Clark's technic so that it could be used with consistent results. Goodpasture and his co-workers,(9, 10, 11, 12) particularly Buddingh, further

developed the technic until it came to be recognized as a reliable experimental procedure. This method was used by Goodpasture and his group and by other workers in the study of various viruses, bacteria, rickettsiae, and spirochetes.

In 1938, Goodpasture(13) mentioned the use of the developing chick membrane as a medium for fungal culture but published no formal communication on this work. Moore,(17, 18) in 1939, and in 1941, for the first time described the use of the chorio-allantoic membrane of the developing chick as a medium for the cultivation and histopathologic study of pathogenic fungi. In 1941, Moore(19) published an additional paper on the use of the developing chick membrane for the cultivation of fungi with particular reference to <u>Histoplasma</u> <u>capsulatum</u>.

TECHNIC FOR INOCULATION OF CHICK EMBRYOS

The technic used in the experiments described below was Goodpasture's modification of Clark's method. Fertile eggs were incubated at 37°C. in an ordinary bacteriological incubator and up to the time of inoculation were turned daily. Care was taken to keep air inside the incubator moist by placing two open beakers of water on the shelf of the incubator. On the day before the eggs were to be used, each egg was candled and the air space was outlined. When the eggs were transluminated during the candling, it was possible to identify the larger blood vessels on the membrane and a cross mark was placed over this area to designate the future site of the window. Eggs with non-viable embryos were discarded.

The shell covering the air sac was cleaned with 80 per cent alcohol. A slit, 4-6mm. in length, was cut in the shell, in order to expose the shell membrane, by means of a hand-electric drill with a circular revolving carburundum disc. The slit was coated with a sterile preparation consisting of three parts paraffin and one part petrolatum. The purpose of the "wax" was to prevent debris and powdered egg shell dust from falling into the air space. The shell membrane was then cut with a sharp

sterile scalpel. Care was taken to insure free access of air into the air sac after the membrane had been cut. It was important that this opening was not occluded, otherwise the chorio-allantoic membrane would not drop away from the shell membrane when the window was cut in the shell.

A window, approximately 1 cm square, was then cut through the shell to the level of the shell membrane at the site previously determined. In doing this the egg was held in the hand and the window was cut with an electric drill. The shell had been washed previously with 80 per cent alcohol and the surface was painted with the sterile "wax". A clean linen towel was rolled in such a way as to form a support for the egg. An alternative method for holding the egg would be by means of a mold of plasticine. The shell membrane under the cut edge of the window was then cut using a sterile scalpel. In cutting the shell membrane great care must be taken not to injure the underlying chorio-allantoic membrane. Once the shell membrane was perforated in almost all cases gravity caused the membrane to fall. The shell window and shell membrane were removed by sterile pointed forceps and discarded. The window was rimmed with sterile petrolatum and a small coverglass was then flamed, allowed to

cool for a moment and placed on the petrolatum rim, partially melting the petrolatum and thus sealing the opening. Two alternatives were open: (1) to inoculate the membrane before sealing with the coverslip, or (2) to seal the egg and place it in the incubator for 24 hours before inoculation. The latter method had the advantage of allowing any embryos whose membranes had been badly traumatized to die before being inoculated, thus eliminating traumatic deaths from the experimental data.

The material to be cultured was placed directly on the membrane with a platinum loop. Once inoculated the eggs were placed in the incubator and observed daily through the coverglass. The embryos were killed after various predetermined intervals following inoculation. When the egg was to be sacrificed, the window was removed and a culture was made on Sabourraud's medium. The edge of the shell was cut down with scissors so that the upper third of the egg was exposed. Zenker's fluid and in some cases alcohol-formalin, was poured over the membrane and allowed to remain for four or five minutes. This method of treatment had the double advantage of giving instant fixation of tissues and of stiffening the membrane _in situ_ so that it could be removed easily. The membrane bear-

ing the growth was then cut away with small iridectomy scissors and floated in sterile-isotonic saline solution. It was then floated on to a small piece of blotting paper in order to prevent the membrane's becoming wrinkled in the fixative. The membrane was fixed in Zenker's fluid or alcohol-formalin, sectioned in paraffin and stained with either phloxine-methylene blue or hematoxylin and eosin, and in some cases with Gram-Wiegert stain.

SPECIES AND STRAINS OF MONILIA

1. A culture of Monilia albicans, which will be designated as Strain 1 in this paper, was obtained on April 1, 1940, from an ulcer of the hand of a male (B.C.H. Out-patient number 612,301). This organism was considered to be the causative agent in the patient's lesion. It was cultured on Sabourraud's medium and gave the usual reactions in sugars for M. albicans (table 10).

2. A strain of Monilia albicans which is designated Strain 2 was obtained at autopsy, (B.C.H. A-42-179), upon culture of the peritoneal cavity of an 81-year old male who died from peritonitis following a perforated chronic duodenal ulcer. This organism was accompanied by a Type 10 pneumococcus and an alpha type of streptococcus. The Monilia was transplanted to Sabourraud's medium and gave the usual reactions in sugars for M. albicans (table 10).

3. A strain of Monilia albicans, which is designated Strain 3, was obtained from a culture of sputum from a male suffering from a lung abscess, (B.C.H. Bacteria specimen B-42-5139). Alpha and alpha prime streptococci, diphtheroids, N. catarrhalis and N. pharyngis sicca were also obtained

from the sputum. The sugar reactions were characteristic of *M. albicans* (table 10).

4. A strain of *Monilia krusei* was obtained from the American Type Culture Collection. This species is usually considered as a non-pathogenic Monilia (table 10).

5. A strain of *Monilia bonordeni* was obtained from the American Type Culture Collection. This species is usually considered as a non-pathogenic Monilia (table 10).

EXPERIMENTAL RESULTS

1. *Monilia albicans*

ORGANISM. Strain 1 of *Monilia albicans* was used to inoculate 31 eggs which had been previously incubated from 10 to 12 days. The eggs were observed daily and were sacrificed at times varying from 1 to 9 days following inoculation. The growth was rapid. By the end of 24 hours there were usually large, well-defined opaque masses of the organism. These were soft, piled-up, gray-white colonies from 0.5 to 4 cm in diameter, their size apparently varying directly with the concentration of the inoculum. These masses of organisms were firmly attached to the membrane and could

not be dislodged without its rupture.

In each case it was possible to recover the organism on Sabouraud's medium or to produce growth by direct transplant to the membrane of a second egg.

The macroscopic growth characteristics of Strains 2 (10 eggs inoculated) and 3 (10 eggs inoculated) were identical with those of Strain 1. In each case growth was recovered on Sabouraud's medium.

HISTOLOGICAL PATHOLOGY. Sections of the chorio-allantoic membrane were taken from the areas in which there was macroscopic growth of *M. albicans*. The histological picture was the same for all strains of *M. albicans* studied as well as for those strains which had been transplanted from membrane to membrane. The colonies themselves were made up of masses of hyphae and spores. At the junction of the colonies and the membrane there was proliferation of the ectoderm. Here the proliferating ectoderm was invaded by a few monocytes, polymorphonuclear leukocytes, and spore forms of the fungi. In some of the sections in which growth had been allowed to progress there were nests of ectodermal-like cells which showed hyperkeratinization and resembled "epithelial pearls". These were particularly

prominent in sections inoculated with Strain 3. The ectodermal layer beneath the more central portion of the colony was either necrotic or could not be identified. A fine eosinophilic granular exudate covered the peripheral surface of the ectoderm and in this exudate could be seen red blood cells, monocytes, polymorphonuclear leukocytes, and cellular debris. In addition there were large masses of fungi present as spore forms and hyphae. The fungi grew in huge masses which extended down into the mesoderm and it was in the mesoderm that the most striking reaction took place.

The mesoderm was greatly thickened in width and this enlargement appeared to be due to edema, to congestion of the blood vessels, and to the presence of large masses of monocytes and granulocytes. Many of the granulocytes were eosinophilic. The fungi grew downward in large projections. At the edge of some of these projections was a margin of ectodermal-like cells giving a rather clear-cut border. Spore forms could be seen in this margin. However, in the majority of the sections this ectodermal-like border was absent and the lesions were much more diffuse and less well defined. There was necrosis in the areas of greatest infiltration. Scattered at the periphery of the masses was seen an occasional giant cell of

the Langhan's type. In some of these giant cells, spores were seen.

The entoderm was relatively uninvolved except in a few areas which were adjacent to marked mesodermal involvement and in these areas there was focal proliferation of the ectoderm together with slight leukocytic infiltration.

2. Monilia krusei

ORGANISM. Monilia krusei was used to inoculate a series of 31 eggs. Growth occurred in each case. The organisms grew in discrete colonies which were piled up and soft, and opaque and gray-white in color. They were loosely adherent to the membrane and could be easily pulled away leaving an intact membrane. Growth was rapid during the first 24 hours and the colonies grew somewhat more slowly during the subsequent 24 hours. By the end of 48 hours the colonies averages 0.5 cm in diameter. The colonies then began to regress and by the end of the next 5 days they had regressed to almost half their former size and were dry and scaley. In some cases, if the process was allowed to go on, the growth would entirely disappear. M. krusei after passage through one membrane was transplanted to other membranes in 11 cases. The colonies were larger but otherwise

were similar to those described above for M. krusei.

HISTOLOGICAL PATHOLOGY. Microscopic sections were taken from areas in which colonies of M. krusei were grossly identified. The periphery of the colonies appeared to be loosely attached to the ectoderm and the colonies were made up for the greater part of masses of spores with only a few hyphae. Much of the ectoderm beneath the colonies showed no reaction but the ectoderm at the center of the colonies usually showed focal areas of proliferation containing occasional spore forms together with a few monocytes and polymorphonuclear leukocytes. The mesoderm for the greater part was uninvolved but focal areas contained a few polymorphonuclear leukocytes and monocytes. The entoderm was not involved.

Sections of membranes inoculated with organisms which had been reactivated and recovered from growth on a previous chick embryo membrane were studied. The reaction was much more marked. Microscopically the colonies did not appear to be as loosely attached as previously described and the ectoderm throughout was invaded by spore forms and infiltrated by monocytes and polymorphonuclear leukocytes. In some areas the ectoderm had sloughed. The mesoderm was thickened and edematous and there was fi-

broblastic proliferation. The blood vessels were distended by nucleated red blood cells. In addition there was diffuse infiltration by polymorphonuclear leukocytes, many eosinophilic cells and a few monocytes. There was no evidence of giant cell formation. In areas in which the mesodermal lesion was most marked there was proliferation of the entoderm.

Sections were taken from membranes upon which there was regression of the colonies. These showed essentially the same histological picture as described above except that the colonies were smaller and the lesions more superficial. In addition there were relatively more monocytes present and there were areas in the mesoderm in which young fibrous tissue had been deposited. Of particular interest were the relatively large numbers of clusters of ectodermal-like cells showing hyperkeratinization. These structures resembled "epithelial pearls".

3. Monilia bonordeni

ORGANISM. A group of membranes of 37 eggs were inoculated with Monilia bonordeni. The colonies grew steadily for 48 hours following inoculation. As in the case of Monilia krusei, the colonies then began to decrease in size so that by the end of 5 days they were definitely smaller and dried

out. However, it was always possible to recover the organism on Sabouraud's medium. At the height of their growth, the colonies were round, gray-white, opaque, discrete, and easily detachable from the membrane. Thirteen eggs were inoculated with M. bonordeni which had been reactivated by passage through previous membranes. The gross cultural characteristics were similar to those described.

HISTOLOGICAL PATHOLOGY. Sections of membrane were taken so as to transect the colonies of M. bonordeni. Colonies were made up of large masses of fungi with many hyphae. The spores were especially prominent. The ectoderm for the most part had sloughed but in those areas in which it remained, it had proliferated and was infiltrated by spore forms, polymorphonuclear leukocytes and monocytes. In sections in which growth had occurred for 48 hours or longer there were groups of ectodermal cells arranged in round nests. These cells showed hyperkeratinization and resembled "epithelial pearls". The mesodermal layer was swollen and diffusely infiltrated by polymorphonuclear leukocytes and mononuclear cells. Many of the granulocytes took an eosin stain. There were spores and hyphae present in the lesion. The blood vessels were congested with red

blood cells. There was slight proliferation of the entoderm in the areas adjacent to the inflamed mesoderm.

Sections for study were taken from membranes which had been inoculated with organisms reactivated and recovered from previous chick embryo membranes. Here the reaction was similar to that previously described but seemed to be more severe. Beneath many of the colonies and extending into the mesoderm were areas of necrosis in which there were masses of polymorphonuclear leukocytes, monocytes, and red blood cells, spore forms and some hyphae.

Sections from membranes in which the growth showed macroscopic regression were studied. The colonies appeared to be but loosely attached to the membrane. There were areas in the mesoderm in which young fibrous tissue had been laid down. The majority of the granulocytes and particularly those taking an eosin stain had disappeared leaving the monocyte as the predominant cell. The ectoderm showed marked proliferation and the lesions seemed to be more superficial than those of the younger stages.

4. PATHOGENICITY

In an effort to secure an approximate estimate of the pathogenicity

of these three species of Monilia, it was decided to determine the mortality among the embryos at an arbitrary period of time. Forty-eight hours was chosen as the figure. It was found that Strain 1 of M. albicans caused a mortality of 37 per cent and that after passage through one membrane, the mortality rose to 45 per cent (tables 1-9). M. albicans, Strain 2, gave a mortality of 70 per cent and Strain 3 of M. albicans gave a mortality of 60 per cent. The mortality figure for M. krusei was 21 per cent while that for M. bonordeni was 26 per cent. When M. krusei and M. bonordeni were passed through one membrane, the mortality rose to 55 per cent and 31 per cent respectively.

DISCUSSION

ORGANISMS. Representative sections of colonies of M. albicans, M. krusei, M. bonordeni were stained by the Gram-Weigert technic. In the colonies of M. albicans on the surface of the chick membrane were thin-walled and slender branching hyphae with chlamydospores at the tips of the branches. In addition there were masses of oval, budding spores which were most numerous around the edges of the colonies. Again, in the lesions produced by this organism, both hyphae and budding forms were present and here the

budding forms were most numerous at the periphery of the lesion. It is interesting to note that a giant cell reaction was stimulated and that some of the budding forms had been enveloped by the giant cells.

In the colonies of *M. krusei* the hyphae appeared as hairlike threads with branching at wider intervals. There were both hyphae and bud forms in the lesions. No chlamydospore-like bodies were present. The budding forms were much larger and more oval than those of *M. albicans*. The proportion of spore forms to hyphae was greater in *M. krusei* than *M. albicans*. In the colonies of *M. bonordeni* the hyphae appeared as thin hairs. No branching or chlamydospores were seen. The budding forms were relatively large, elongated and quite numerous. Both forms were present in the lesion though the budding forms predominated. The findings were consistent enough to permit the identification of each organism by the appearance of the hyphae and spores.

PATHOLOGY. The lesions produced by the three organisms were essentially the same and differed in degree rather than kind. The lesions produced by *M. albicans* were more severe than those produced by the other two. The *M. albicans* produced more necrosis and the lesions were of greater depth.

Giant cell formation was seen in lesions produced by *M. albicans.* No giant cells were seen in lesions produced by the first and second generations of *M. krusei* and the first generation of *M. bonordeni.* It is of interest to note that the lesions of *M. bonordeni* were more severe and that there was giant cell formation after the passage of the organism through one membrane.

PATHOGENICITY. The mortality of the embryos after forty-eight hours using fungus obtained from culture averaged 38-70 per cent for *M. albicans;* 21 per cent for *M. krusei;* and 26 per cent for *M. bonordeni.* Confirmation of the fact that the lesions produced by *M. albicans* were more severe is that it was not uncommon for colonies of *M. krusei* and *M. bonordeni* to regress in size or even disappear. The mortality figures deserve comment. It is interesting to note that Strain 1 of *M. albicans* gave mortality of only 38 per cent while both Strains 2 and 3 caused a mortality of 70 per cent and 60 per cent respectively. Also, after the passage of Strain 1 through an egg membrane, the mortality rose to 45 per cent. It is possible that the discrepancy between the low mortality rate of Strain 1 and of Strain 2 and 3 is due to the fact that Strain 1 had been carried on Sab-

ouraud's medium for a period of years while Strains 2 and 3 were freshly isolated. Further confirmation of this suggestion is the increase of the mortality rate from 38 to 45 per cent after the passage of the fungus through but one egg membrane. It is also interesting to note the increase in mortality rate and severity of the lesions after the passage of M. krusei and M. bonordeni through membranes. It would seem that the chorio-allantoic membrane of the developing chick would offer a readily available means for increasing the virulence of these organisms.

Moore(18) was impressed with the formation of the epithelial pearls in the mesoderm after the inoculation of the membrane with M. albicans. He states "this process was analogous in all respects to that in infection of human epithelium with the same organism". * These structures were found in the present series but were present not only in the membranes inoculated with M. albicans but also in those membranes inoculated with M. krusei and M. bonordeni. Emmart and Smith(6) produced similar "pearls" by injection of tuberculin and by the implantation of tubercle bacilli on the chorio-allantoic membrane. Moore(19) describes ectodermal pearls in the

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* M. Moore, "The Chorio-allantoic Membrane of the Developing Chick as a Medium for the Cultivation and Histopathologic Study of Pathogenic Fungi". Am. J. Path., 1941, 17, p.112.

mesoderm after the implantation of Histoplasma capsulatum. Canat and Opie(3, 4) demonstrated proliferation of ectodermal cells with the formation of papilla-like projections with abnormal keratinization of cells following the introduction of carbon particles and turpentine. It would seem that the formation of pearls and the keratinization and hyperplasia of ectoderm is not a specific reaction to M. albicans but rather is a non-specific reaction on the part of the membrane to any type of irritant.

SUMMARY

1. A comparative study of the cultural characteristics of three strains of Monilia albicans, one strain of Monilia bonordeni, and one strain of Monilia krusei on the chorio-allantoic membrane of chick embryos has been made.

2. The lesions in the membrane produced by the various species are described and discussed.

3. It is suggested that passage through the chorio-allantoic membrane causes the virulence of these organisms to be increased.

4. It is concluded that the pathological reactions to these organisms are similar but that the pathogen, Monilia albicans, causes lesions

which are much more severe in degree than those of the questionable nonpathogens, Monilia bonordeni and Monilia krusei.

TABULATION OF EXPERIMENTAL DATA

TABLE 1

Mortality of Chick Embryos after 48 Hours

Monilia albicans	
Strain 1	
A. Obtained from culture	38 per cent
B. After membrane passage	45 per cent
Strain 2	
A. Obtained from culture	70 per cent
Strain 3	
A. Obtained from culture	60 per cent
Monilia krusei	
A. Obtained from culture	21 per cent
B. After membrane passage	55 per cent
Monilia bonordeni	
A. Obtained from culture	26 per cent
B. After membrane passage	31 per cent

SYMBOLS--In tables 2-10 the following symbols have been used:

Growth

+ = Slight
++ = Moderate
+++ = Extensive
++++ = Luxuriant
R= Regression

State of Embryo

D = Dead
A = Alive

Acid and Gas

A sl = Slightly acid
G numerals = percentage of gas

TABLE 2

Monilia albicans, Strain 1 (Obtained from culture)

Number of Egg	Growth			Final Growth		Growth Recovered	Microscopic Sections
	48 hours	120 hours	Final	Time(Days)	State of Embryo		
1	++	+++	++++	6	A	Yes	Yes
2			++	1	D	Yes	No
18	++		++	3	D	Yes	Yes
19	+++		+++	3	D	Yes	Yes
21	+		+	3	D	Yes	Yes
22	++	+++	+++	9	A	Yes	Yes
46	++	+++	+++	5	D	Yes	No
47	++	+++	+++	5	A	Yes	Yes
48	++	+++	+++	5	A	Yes	No
50	++	+++	+++	5	D	Yes	Yes
51			+	1	D	Yes	No
126			+++	1	D	Yes	No
127	+++		+++	2	D	Yes	No
128	++		+++	3	D	Yes	No
129			++	1	D	Yes	No
130			++	1	D	Yes	No

TABLE 3

<u>Monilia</u> <u>albicans</u>, Strain 1 (After membrane passage)

Number of Egg	Cultured from Egg	Growth			Final	Growth	Growth Recovered	Microscopic Sections
		48 Hours	120 Hours	Final	Time (Days)	State of Embryo		
20	1	++	+++	+++	7	D	Yes	Yes
23	1	++	+++	+++	7	D	Yes	Yes
33	1	+	++	++	5	D	Yes	Yes
34	1	+	+	+	9	D	Yes	Yes
35	1	+	++	++	5	D	Yes	No
36	1	+	++	+++	5	D	Yes	Yes
37	1	+	++	+++	5	D	Yes	Yes
38	1	++	++	+++	5	D	Yes	No
131	1	++	++	+++	2	D	Yes	No
132	1	++	++	+++	2	D	Yes	No
133	1			++	1	D	Yes	No
134	1			++	1	D	Yes	No
135	1	++		++	2	D	Yes	No
136	1			++	1	D	Yes	No
137	1	+++		+++	2	D	Yes	No

TABLE 4

Monilia albicans, Strain 2 (Obtained from culture)

Number of Egg	Growth			Final Growth			
	48 hours	120 hours	Final	Time (Days)	State of Embryo	Growth Recovered	Microscopic Sections
58	++	+++	+++	7	D	Yes	Yes
59	++	+++	++++	7	D	Yes	No
60			+	1	D	Yes	No
61	++		++	2	D	Yes	Yes
62	+		+	2	D	Yes	Yes
149			+	1	D	Yes	No
150	++		+++	3	D	Yes	No
151	++		++	2	D	Yes	No
152			+	1	D	Yes	No
153			+	1	D	Yes	No

TABLE 5

Monilia albicans, Strain 3 (Obtained from culture)

Number of Egg	Growth			Final Growth			
	48 hours	120 hours	Final	Time (Days)	State of Embryo	Growth Recovered	Microscopic Sections
63	+	++	++	7	A	Yes	No
64	++	+++	++++	14	A	Yes	Yes
65	++	+++	++++	14	D	Yes	Yes
66	++	+++	++++	7	A	Yes	Yes
67			+	1	D	Yes	No
143	++		++	2	D	Yes	No
144	++		++	2	D	Yes	No
145			++	1	D	Yes	No
147	+		+	1	D	Yes	No
148	++		++	1	D	Yes	No

TABLE 6

Monilia krusei (Obtained from culture)

Number of Egg	Growth			Final Growth			
	48 hours	120 hours	Final	Time (Days)	State of Embryo	Growth Recovered	Microscopic Sections
68	++	R	+	12	A	Yes	Yes
70	++	R	+	10	A	Yes	Yes
71	+		+	12	D	Yes	No
72	+++	R	+	12	D	No	No
73	++	R	+	7	D	Yes	Yes
74	+++	R	+	12	A	Yes	Yes
96	++	R	+	10	A	Yes	Yes
97	++	R	+	10	A	Yes	Yes
98	+	R	+	2	A	Yes	No
99	++	R	+	2	D	Yes	Yes
154			+	1	D	Yes	No
155	++	R	+	4	D	Yes	No
156			+	1	D	Yes	No
187			++	1	A *	Yes	Yes
188	+		+	2	D	Yes	No
189	++	R	+	6	A	Yes	Yes
197	++		++	2	A	Yes	Yes
198	++	R	+	6	A	Yes	Yes
199	++		++	3	A	Yes	Yes
200	++	R	Dis-appeared	6	A	No	Yes

* Killed for 24 hour section. Not considered in mortality rate.

TABLE 7

Monilia krusei (After membrane passage)

Number of Egg	Culture from Egg	Growth 48 Hours	Growth 120 Hours	Growth Final	Final Growth Time (Days)	Final Growth State of Embryo	Growth Recovered	Microscopic Sections
108	73	++		+++	7	D	Yes	Yes
110	74	++		+++	10	A	Yes	Yes
111	68	++		++	2	D	Yes	Yes
112	68	++		++	2	D	Yes	Yes
113	70	++		++	2	D	Yes	Yes
114	70	++		+++	10	A	Yes	Yes
123	71	++		+++	10	A	Yes	Yes
125	70	++		++	2	D	Yes	Yes
168	68			++	1	D	Yes	No
169	73	++		++	4	D	Yes	No
170	73			++	1	D	Yes	No

TABLE 8

Monilia bonordeni (Obtained from culture)

Number of Egg	Growth 48 hours	120 hours	Final	Final Growth Time (Days)	State of Embryo	Growth Recovered	Microscopic Sections
75	+		+	2	D	Yes	Yes
76	+		+	2	D	Yes	Yes
77	+		+	2	D	Yes	Yes
78	++	R	++	10	A	Yes	Yes
79	++	R	++	12	A	Yes	Yes
80	++	R	++	7	D	Yes	Yes
81	++	R	+	12	D	Yes	Yes
82	++	R	++	10	A	Yes	Yes
100	++		++	2	D	Yes	Yes
101	+++	R	++	10	A	Yes	Yes
102	++	R	+	7	D	Yes	Yes
103	++	R	+	4	D	Yes	Yes
177	++	R	+	4	D	Yes	No
178	++	R	+	4	D	Yes	No
179	++		++	4	D	Yes	No
180	++		++	2	D	Yes	No
181			+	1	D	Yes	No
190			++	1	A	Yes *	Yes
191	++		++	2	A	Yes	Yes
192	++	R	+	6	A	Yes	Yes
193	++	R	+	6	A	Yes	Yes
194	++	R	+	6	A	Yes	Yes
195	++		++	3	A	Yes	Yes
196	++	R	+	6	A	Yes	Yes

*Killed for 24 hour section. Not considered in mortality rate.

TABLE 9

<u>Monilia</u> <u>bonordeni</u> (After membrane passage)

Number of Egg	Culture from Egg	Growth						
		48 Hours	120 Hours	Final	Time (Days)	State of Embryo	Growth Recovered	Microscopic Sections
115	77	+	++	+	4	D	Yes	Yes
116	77	+		+	2	D	Yes	Yes
117	75	++		++	4	D	Yes	Yes
118	75	++	R	+	10	D	Yes	Yes
119	80	++		++	4	D	Yes	Yes
120	80	++		++	10	A	Yes	Yes
121	78	++		++	2	D	Yes	Yes
122	78	++		+++	10	D	Yes	Yes
182	81	++		++	4	D	Yes	No
183	81	++		++	4	D	Yes	No
184	81	++		++	2	D	Yes	No
185	81	++		++	4	D	Yes	No
186	81	++		++	2	D	Yes	No

TABLE 10

Differentiation of Monilias Used with Sugar Fermentation *

After 3 days

Species	Dextrin	Dextrose	Galactose	Inulin	Lactose	Levulose	Maltose	Mannose	Saccharose
M. albicans, Strain 1	-	A-sl G-20	A-sl	-	-	A G-bubbles	A	A-sl G-20	A-sl
M. albicans, Strain 2	-	A-sl G-60	A-sl	-	-	A G-100	A G-100	A-sl G-50	A-sl
M. albicans, Strain 3	A-sl	A-very sl G-50	-	-	-	A G-100	A G-66	A G-30	A-sl G-50
M. krusei	-	A-sl G-60	-	-	-	A G-25	-	A-sl G-50	-
M. bonordeni	-	A-sl G-60	A-sl G-bubbles	-	-	A G-100	A G-15	A-sl G-50	A-sl G-60

After 6 days

Species	Dextrin	Dextrose	Galactose	Inulin	Lactose	Levulose	Maltose	Mannose	Saccharose
M. albicans, Strain 1	-	A G	A-sl	-	-	A G-10	A Gbubbles	A G-10	A-sl
M. albicans, Strain 2	-	A G	A-sl	-	-	A G-80	A G-75	A-sl G-25	A-sl
M. albicans, Strain 3	-	A G	-	-	-	A G-80	A G-75	A G-10	A G-15
M. krusei	-	A G	-	-	-	A G-100	-	A-sl G-33	A-sl G-3
M. bonordeni	-	A G	A-sl G-10	-	-	A G-80	A G-20	A G-40	A-sl G-33

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* Adapted from a Practical Classification of the Monilias by Martin, Jones, Yao and Lee (J. Bact., 1937, 34, 110-111).

BIBLIOGRAPHY

1. Askanazy, M. Pathologische Anatomie, Aschoff, L. Gustav Fischer, Jena, 1923, 187.

2. Beguelin. Memoire sur l'art de couver les oeufs ouverts. Hist. de l'Acad. de Berlin, 1749, p. 71. (Cited by Gerlach, 8).

3. Canat, E. H., and Opie, E. L. Inflammation in embryonic life. I. Changes produced by particulate matter and by a chemical agent. Am. J. Path., 1943, 19, 371-383.

4. Canat, E. H., and Opie, E. L. Inflammation in embryonic life. II. Infection of chick embryos with avian tubercle bacilli. Am. J. Path., 1943, 19, 385-394.

5. Clark, E. R. Technique of operating on chick embryos. Science, 1920, 51, 371-373.

6. Emmart, E. W., and Smith, M. I. The growth and effects of the tubercle bacillus on the chorio-allantoic membrane of the chick embryo. A method for studies in chemotherapy. Pub. Health Rep., 1941, 56, 1277-1286.

7. Gay, F. P., and Thompson, R. Attempts to cultivate vaccine virus in the growing chick embryo. Proc. Soc. Exper. Biol. and Med., 1929, 26, 556-559.

8. Gerlach, L. Über neuere methoden auf dem gebiete der experimentellen embryologie. Bardeleben, Anatomischer Anzeiger, 1886, vol 2, no. 18-19, 583.

9. Goodpasture, E. W.; Woodruff, A. M., and Buddingh, G. J. The cultivation of vaccine and other viruses in the chorio-allantoic membrane of chick embryos. Science, 1931, 74, 371-372.

10. Goodpasture, E. W., Woodruff, A. M., and Buddingh, G. J. Vaccinal infection of chorio-allantoic membrane of the chick embryo. Am. J. Path., 1932, 8, 271-282.

11. Goodpasture, E. W. Use of embryo chick in investigation of certain pathological problems. South. M. J., 1933, 26, 418-420.

12. Goodpasture, E. W., and Anderson, K. The problem of infection as presented by bacterial invasion of the chorio-allantoic membrane of chick embryos. Am. J. Path., 1937, 13, 149-174.

13. Goodpasture, E. W. Some uses of the chick embryo for the study of infection and immunity. Am. J. Hyg., 1938, 28, 111-129.

14. Juan, C. and Staub, A. Étude sur la peste aviaire. Ann. Inst. Pasteur, 1920, 34, 343-357.

15. Levaditi, C. La spirillose des embryons de poulet dans ses rapports avec la tréponémose héréditaire de l'homme. Ann. Inst. Pasteur, 1906, 20, 924-938.

16. Martin, D. S., Jones, C. P., Yao, K. F. and Lee, Jr. L. E. Practical classification of monilias. J. Bact., 1937, 34, 99-129.

17. Moore, M. The chorio-allantoic membrane of the developing chick as a medium for the cultivation and histopathologic study of pathogenic fungi. Science, 1939, 89, 514-515.

18. Moore, M. The chorio-allantoic membrane of the developing chick as a medium for the cultivation and histopathologic study of pathogenic fungi. Am. J. Path., 1941, 17, 103-120.

19. Moore, M. Histoplasma capsulatum; its cultivation on chorio-allantoic membrane of developing chick and resultant lesions. Am. J. Trop. Med., 1941, 21, 627-643.

20. Murphy, J. B. Studies in tissue specificity. II. The ultimate fate of mammalian tissue implanted in the chick embryo. J. Exper. Med., 1914, 19, 181-186.

21. Peebles, F. Some experiments on the primitive streak of the chick. Arch. f. Entwcklngsmechn, 1898, 7, 405-429.

22. Rettger, L. F. The bacteriology of the hen's egg with special reference to its freedom from microbic invasion. Centralbl. f. Bakt., Pt. II, 1913-14, 39, 611-624.

23. Rous, P., and Murphy, J. B. Tumor implantations in the developing embryo. Experiments with a transmissible sarcoma of the fowl. J. A. M. A., 1911, 56, 741-742.

24. Scymkiewicz. Beitrag zur der lehre von den künstlichen missbildungen am hühnerei. Wien. Sitzungber., 1815, 62, 139. (Cited by Gerlach, 8).

25. Stevenson, H. N. Growth of tumors in the chick embryo. J. Canc. Res., 1918, 3, 63-74.

26. Wolff, M., and Israel, J. Ueber reincultur des Actinomyces und seine

uebertragbarkeit auf thiere. Virchows Arch. f. path. Anat., 1891, 126, 11-59.

27. Woodruff, A. M., and Goodpasture, E. W. The susceptibility of the chorio-allantoic membrane of chick embryos to infection with the fowl-pox virus. Am. J. Path., 1931, 7, 209-222.